

Title: Hypoalgesic effects of strength exercise-induced fatigue in healthy individuals.

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STUDY PROTOCOL

ABSTRACT

Strength exercise is a type of physical activity that aims to apply force against resistance. This produces a perception of subjective effort and fatigue during and after its performance. Exercise-Induced Hypoalgesia (HIE), one of the most relevant effects of exercise, is a phenomenon by which the perception of pain is reduced during and after an exercise session. Its mechanisms are not yet understood in detail, as well as the influence of fatigue induced by force exercise on HIE.

The objective of this research project is to determine whether fatigue and perceived exertion in strength exercise are a relevant factor in HIE in healthy subjects. To this end, a randomized repeated-measures crossover trial in blinded healthy subjects is designed. Two endurance exercise protocols will be carried out with the aim of producing different levels of fatigue and a control protocol, over 3 different sessions separated by 7 days. The demographic, PAR-Q+, IPAQ-SF, and Wellness questionnaires will be used to assess compliance with study participation criteria, familiarity with strength training, and management of fatigability and blinding success. To determine the HIE, the threshold of pressure pain and pressure-evoked pain will be used. To determine fatigue, a maximum isometric strength test of knee extension will be performed, while to determine the perception of subjective effort, the OMNI-RES scales and the ERF scale will be used.

Regarding the statistical analysis, an ANOVA repeated measures test will be performed to evaluate the changes in pain thresholds and their relationship with the level of fatigue achieved. In addition, the correlations between the different variables explored will be explored to better understand their interaction with the HIE.

The findings of this study will contribute to clarifying the knowledge about exercise-induced analgesia, allowing to improve the prescription in clinical and sports contexts, optimizing its analgesic effects. Understanding the mechanisms of HIE and its relationship with fatigue can contribute to the recommendation of strength exercise in the prevention and treatment of pain.

INTRODUCTION

Exercise is defined as a type of planned and structured physical activity aimed at improving or maintaining various components of physical fitness (1). It is termed

therapeutic exercise when the ultimate goal is to reduce disability, improve functional capacity, and physical fitness, both preventively and during the recovery process of a patient whose movement and activities of daily living have been affected (2).

Therapeutic exercise must be prescribed systematically, individually, and specifically, from a biobehavioral approach, considering practical parameters such as frequency, intensity, type of exercise, duration, and volume. Biobehavioral analysis of the patient and their environment, goal setting, and therapeutic education are also fundamental (3).

Resistance or strength exercise consists of applying force against a resistance to achieve muscle contraction. The goal of this type of training is usually to work on muscle strength and often generate hypertrophy. Key variables of strength exercise include frequency, type of exercise, duration, training volume, and intensity, among others. Depending on the movement of the body structures involved, strength exercises can be static if they do not move, or dynamic if they do. An example of a dynamic strength exercise is the leg extension. This is a monoarticular exercise consisting of a bilateral open kinetic chain knee extension performed on a guided machine in a single plane.

For the dosage of dynamic strength exercises, the number of sets, repetitions, rest time between sets, and cadence or contraction times during the exercise are usually monitored. Performing strength exercise produces a specific level of neuromuscular fatigue. Fatigue can be defined as a reduction in the capacity of the neuromuscular system to generate force, regardless of the level of force required (4,5). It is common for strength work to reach very high intensities near neuromuscular failure, which prevents you from continuing with the exercise (1).

In dynamic strength exercises, intensity can be estimated using external load through the percentage of the One-Repetition Maximum (%1RM) or internal load, using subjective stress perception, using the Effort Perception Scale (RPE) (3). Perceived exertion during exercise is a cognitive interpretation caused by various metabolic changes in the musculoskeletal, cardiopulmonary, and central nervous systems associated with exercise (1). An increase in perceived exertion is related to muscle fatigue or fatigability, which also involves a loss of the capacity to apply force induced by exercise through the interaction of peripheral and central mechanisms (6).

Exercise is able to reduce pain perception during or after an exercise session, a phenomenon known as Exercise-Induced Hypoalgesia (EIH) (7). EIH, in addition to reducing the perception of pain intensity or perceived unpleasantness, can also manifest as an increase in pain threshold or pain tolerance, in both patients with pain and asymptomatic individuals (8). EIH is usually assessed immediately after exercise, up to

15 minutes after the cessation of activity (6), although some studies report that these results are maintained even 60 minutes later (7).

However, the exact efficacy of EIH in individuals with chronic musculoskeletal pain is currently unknown due to the low quality of current studies and their variability regarding clinical conditions and exercise dosage (5). The mechanisms responsible for EIH in humans are also not precisely known. Animal studies have found that the administration of opioid antagonists, such as naloxone, attenuates the hypoalgesic response to exercise. This result indicates that EIH is partly mediated by the endogenous opioid system; however, since EIH was not completely abolished, it is proposed that part of its effect is non-opioid dependent (9–11). Therefore, although the opioid system is considered to be one of the primary mechanisms of EIH (12), it could be due to multiple analgesic mechanisms.

An alternative to opioid hypoalgesia is that mediated by the endocannabinoid system. A study conducted with university students found that moderate-intensity exercise significantly increased anandamide concentration in blood plasma (13,14). This increase could have implications for EIH because activation of the endocannabinoid system reduces pain perception (14,15) and influences emotional and cognitive processes (14,16). Furthermore, unlike the suppression of pain neurotransmission by opioids, the endocannabinoid system influences nociception not only at the central level but also in the peripheral nervous system (14,15,17).

There is no consensus on the optimal dose of exercise required to produce EIH (18). It is known that a single session of exercise can reduce pain sensitivity in healthy individuals (8). Moreover, it has been shown that varying exercise parameters, such as duration or whether the exercise is performed intermittently or continuously, influences the emergence of mostly opioid or mostly non-opioid EIH (9,10). The magnitude of EIH seems to vary according to the modality, dose, and intensity of the exercise performed (7,19–21), as well as the assessment methodology, such as the pain assessment tool (7,18), the location (7,22), or the timing of the measurement (7,23–25). Nevertheless, the response to exercise is highly variable between individuals and between days. In some cases, hyperalgesia may occur after exercise, but most healthy individuals show EIH at some point after exercise (8).

Recently, EIH has been reported more significantly after high-fatigue exercise, specifically at the local level. That study used protocols that only sought to achieve a certain RPE and whose HIE measure was only the pain thresholds at pressure (PPT) (26). On the other hand, in the protocol that produced low fatigue, greater changes in

EIH were observed at a distance in subjects with efficient endogenous systems; additionally, subjects with lower fear of pain achieved higher levels of hypoalgesia than those who were afraid (26). However, further research is needed to precisely understand the relationship between fatigue and exercise-induced hypoalgesia.

JUSTIFICATION AND OBJECTIVES

Understanding the physiological mechanisms of exercise on pain and the parameters that favor the appearance of EIH is fundamental for proposing new recommendations and adjusting current ones for healthy individuals as a preventive measure, and for individuals with pain as part of functional rehabilitation process. Knowing EIH physiology, in addition to improving our current recommendations, can help minimize adverse effects associated with exercise.

EIH has been reported in isometric, resistance, and aerobic exercises, although there are conflicting results on which exercise modality is most effective in terms of analgesia (7,27). Interestingly, in aerobic exercise, intensity does not seem to determine the EIH level (28). In contrast, strength exercise intensity may influence EIH, with moderate intensity appearing to have the greatest effect, followed by high intensity. On the other hand, low intensity does not seem to have significant effects in resistance exercises.

Therefore, current recommendations for EIH in strength exercise involve moderate-to-high intensity. These observations lead to the theoretical proposal that it may be necessary to recruit high-threshold motor fibers for EIH to occur (27), although no studies have confirmed this. Recent studies applying different strength training intensities did not find EIH after exercise. However, results could be biased by the application of a noxious stimulus when measuring Conditioned Pain Modulation (CPM) (29).

Furthermore, most studies evaluated intensity based on external load through the percentage of the One-Repetition Maximum (%1RM) without considering internal load of exercise by monitoring subjective variables such as RPE, which could have greater practical and clinical applicability. No study has directly related EIH to exercise-induced fatigue using objective variables like isometric strength and RPE as a subjective variable. Nor have studies compared EIH in resistance exercises using different distributions that alter exercise training density.

This justifies the present research project, whose **main objective** is to determine whether fatigue and perceived effort in strength exercise are relevant factors for EIH in healthy subjects.

As **secondary objectives** we propose:

- Exploring a possible association between previous physical activity levels and familiarity with strength training regarding the magnitude of EIH obtained and the onset of fatigue during strength exercise.
- Exploring whether the onset and magnitude of EIH are associated with various subject variables (prior fatigue state, sleep quality, general muscle status, pain, stress level, and mood).
- Exploring a possible association between an individual's previous fatigue state, sleep quality, general muscular state, pain, stress level, and mood with the onset of fatigue during strength exercise.

Overall, this project aims to understand the role of fatigue in high-intensity strength exercise, which would help to better understand the mechanisms and variables that influence EIH. Consequently, it will directly impact clinical practice by advising whether strength exercise should be fatiguing or not, considering potential adverse effects.

RESEARCH TYPE AND DESIGN

An experimental randomized crossover trial with repeated measures in blinded healthy subjects is proposed. After reading the information sheet, receiving appropriate explanations, and signing the informed consent (Annex 1), participants will perform the "leg extension" strength exercise on a guided machine, carrying out different protocols that aim to produce different levels of fatigue, which will be monitored objectively, through a maximum isometric strength test, and subjectively, by patient reports. Measurements will be conducted to determine whether an Exercise-Induced Hypoalgesia (EIH) response occurs and whether it depends on the level of fatigue achieved in each protocol. These measurements will be taken both locally and peripherally to relate the results to the different endogenous systems that may intervene in EIH.

This research proposal consists of a **crossover trial** with repeated measures where all subjects attend three different exercise sessions separated by at least one week: one control session and two interventions with different degrees of fatigue, which are detailed in their corresponding section.

TARGET POPULATION AND SELECTION CRITERIA

Participant recruitment will be carried out verbally and online, with publications on social networks, based on the following criteria for participation:

- **Inclusion Criteria**

- Men and women.
- Young adults, aged between 18 and 45 years.
- They will be healthy, asymptomatic and physically active people.
- Commitment to completing all three sessions and not withdrawing from the study.

- **Exclusion Criteria**

- Presence of acute pain episodes in any body region.
- Presence of chronic pain or a history of recent chronic pain resolved within the last 6 months.
- Presence of severe pathology or inability to perform moderate-to-intense physical activity.
- Consumption of analgesics, antipyretics, non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, opioids, or similar drugs within 24 hours prior to a session.
- Consumption of alcohol or other psychoactive substances, caffeine, and other stimulants within 24 hours prior to a session.
- Performance of fatiguing physical activity or strength exercise within 24 hours prior to a session.
- Physical condition preventing normal study performance, including obtaining a 10RM greater than 75 kg or less than 5 kg in the leg extension exercise.
- The use of simultaneous pharmacological treatments or substances that could interfere with the results or pose a risk to the subject, as well as various pathologies that could influence the outcomes.

The selection will focus on the university community of the Miguel Hernández University of Elche, specifically students from the Faculty of Medicine at the Sant Joan d'Alacant Campus and users of the gyms at the UMH Sports Center in Sant Joan.

DESCRIPTION OF THE INTERVENTION PROCEDURE

All participants will attend three different sessions. The first will always be a control session, and the remaining two will be intervention sessions, which will be randomized.

For the intervention, the leg extension strength exercise will be performed on a machine. The equipment consists of a padded seat, two adjustable padded arms (one to limit hip movement and another against which the push is performed), and a pulley system connected to metal weight plates. For the execution, the subject sits on the machine with knees at 90° flexion, and the machine arms are adjusted over the distal portion of the thighs and legs, respectively. To perform the exercise, the subject extends the knees against the resistance provided by the machine, using an individualized load for each participant based on their 10RM (maximum load for 10 repetitions), the measurement of which is explained below.

Due to its characteristics, leg extension is a strength exercise that allows us to have greater control during execution and specific evaluation of the effects of strength exercise on EIH, minimizing the impact of other variables associated with more complex movements. Furthermore, it is an exercise that is easy to learn and that, due to its low impact and great stability, is a suitable exercise to be used in the intervention of this study.

During the exercise, a metronome will be used at 60 beats per minute that will mark the cadence of the concentric and eccentric phases of the exercise, which will be 1:2 in all sessions. Any changes in execution tempo due to loss of rhythm, fatigue, or inability to complete the set will be recorded.

In the **Control session**, subjects will be familiarized with the Rate of Perceived Exertion (RPE) and Repetitions in Reserve (RIR) scales. Anthropometric measurements of the lower limbs will be taken using a tape measure from the anterior superior iliac spine to the internal malleolus of the tibia as it has proven to be a reliable measurement of the length of the lower limb in healthy people (30).

To determine the external load used in the exercise, the 10RM test will be carried out following the lines of the study by Santana et al., 2021 (31), which coincides with the methodology of previous research (32-34). For subjects familiar with the exercise, the initial load will be estimated based on their usual training weights. For unfamiliar subjects, 20% of their body weight will be used as a starting load to ensure safety. If 10 repetitions cannot be completed or if more than 10 are possible, the load will be adjusted by 1–10 kg, and another attempt will be made after 5 minutes of rest until the target weight is reached (34). The objective of the 10RM test is to perform 10 consecutive repetitions reaching failure in the last one, so that it is impossible for the subject to perform one more repetition with that weight.

For the 10RM test, we will add the use of the metronome so that the concentric-eccentric contraction cadence provided for in the study protocols is respected.

The intervention (Sessions 2 and 3) consists of two protocols with identical training volume (30 total repetitions), the same duration of the exercise (90 seconds of time under tension), the same external load (100% of the 10RM), and relative rest between sets (180 seconds) but different total rest time (360 seconds in the AF session vs 900 seconds in the BF session) and different total duration of the exercise (450 seconds in the AF session vs 990 seconds in the BF session). The exercises will be performed in all cases with a concentric-eccentric contraction cadence of 1:2. Subjects will be instructed to control breathing during the exercises, inhaling during the concentric phase and exhaling during the eccentric phase. These protocols are a modification of the protocols used in Costa's study (35), in which they equalize volume, external load, and total duration of breaks and activity. It was decided to equalize the relative rest time between sets to further differentiate the sessions from each other, since in their study they did not see differences in terms of performance loss in countermovement jumps (CMJ), although they did see differences in terms of perceived internal load and subjective evaluation of effort (RPE).

The warm-up will be the same for both protocols, and prior to the control intervention, with the exception that the last one will be carried out without load. This warm-up is the same as that used in the protocols (35) and consists of 2 sets of 15 repetitions with 50% of the 10RM with a 2-minute rest in between.

Below is a summary description of the different sessions:

- **Initial Control Session (C):** the subjects will perform the protocol of the Low Fatigue session (explained below) but without load and without any type of resistance, on the same leg extension machine.

- **Low Fatigue Session (LF):** Subjects will perform 6 sets of 5 repetitions with 180 seconds of rest between sets, using an external load of 100% of the 10RM and a 1:2 cadence of concentric-eccentric contraction.
- **High Fatigue Session (HF):** Subjects will perform 3 sets of 10 repetitions with 180 seconds of rest between sets, using an external load of 100% of their 10RM and a 1:2 cadence of concentric-eccentric contraction.

RANDOMIZATION AND BLINDING

The first session will always be the control session, and the other two intervention sessions will follow a randomized order, and the specific objective of each protocol will not be disclosed to maintain blinding and prevent bias.

For the randomization of the order of the sessions, a proprietary software developed in R will be used, which will randomly assign each participant one of the two possible orders:

- **Sequence A:** Session 1 (Control) → Session 2 (HF Intervention) → Session 3 (LF Intervention).
- **Sequence B:** Session 1 (Control) → Session 2 (LF Intervention) → Session 3 (HF Intervention).

Due to the limitations of blinding in this type of study, they will be administered a questionnaire to evaluate the success of blinding (Annex 3) following the recommendations of Kolahi et al. (36). This questionnaire will be administered to the subjects at the end of the activity in each of the sessions without reminding them of the results indicated in the previous sessions to reduce biases.

CLINICAL RESEARCH DEVELOPMENT AND RESPONSE ASSESSMENT

The study will be conducted over 3 different sessions (1 control and 2 interventions), separated by at least 7 days. All of them will be carried out at the Sports Center of the Sant Joan Campus of the Miguel Hernández University of Elche (Avenida de la Cadena s/n, San Juan de Alicante, Valencian Community, Spain), where the leg extension machine is located, model SALTER M-2082, which operates through a pulley mechanism and allows the weight to be adjusted from 5 to 75 kg, with the possibility of adding small

extra weights of 0.5kg, 1kg, and 2.5kg for greater precision with the external load used, up to 100kg as specified by the manufacturer.

Before the sessions, participants will be reminded to arrive in sportswear, preferably shorts; they must not consume stimulants such as coffee or similar, analgesic or anti-inflammatory medication, nor have performed physical exercise on the 24h prior to attending each session. Therefore, to begin each session, it must be ensured that all subjects meet the criteria for participation in the study.

In the first session (control), after being informed about the study and signing the informed consent (Annex 1), they will complete a questionnaire on descriptive variables and compliance with the selection criteria in all sessions (Annex 2). Following this, the Physical Activity Readiness Questionnaire+ (PAR-Q+) will be administered to ensure that the subjects are fit to perform the exercise program safely (37). Complementarily, the short version of the International Physical Activity Questionnaire (IPAQ-SF) will be carried out, which will allow us to classify the subjects based on their physical activity performance (38). To monitor the state of the subjects in each of the study sessions, they will also fill out the Wellness questionnaire, a tool to assess the state of fatigue, sleep quality, general muscle state, pain, stress level, and mood in athletes (39).

The first session will continue with the baseline measurement of algometry followed by the maximal isometric strength test. These will be performed on the same leg extension machine prior to the intervention. The control intervention will consist of performing the LF protocol, but without load; the patient will perform the exercise in the air, without the resistance of the machine. Algometry and the maximal isometric strength test will be measured again immediately after and at 30 minutes post-intervention. Once finished the control exercise protocol, the algometry measurements, isometric strength at both points in time and assessing the sRPE scale, the 10RM test will be performed to determine the load to be used in the protocols for sessions 2 and 3 without this altering the perception of pain or the sensation of fatigue from the control session.

The experimental sessions (2 and 3) will have a randomized order and will be identical to the control session, with the exception that the subjects will perform the HF and LF training protocols.

Below, the flow of the development of the experimental design is visually represented:

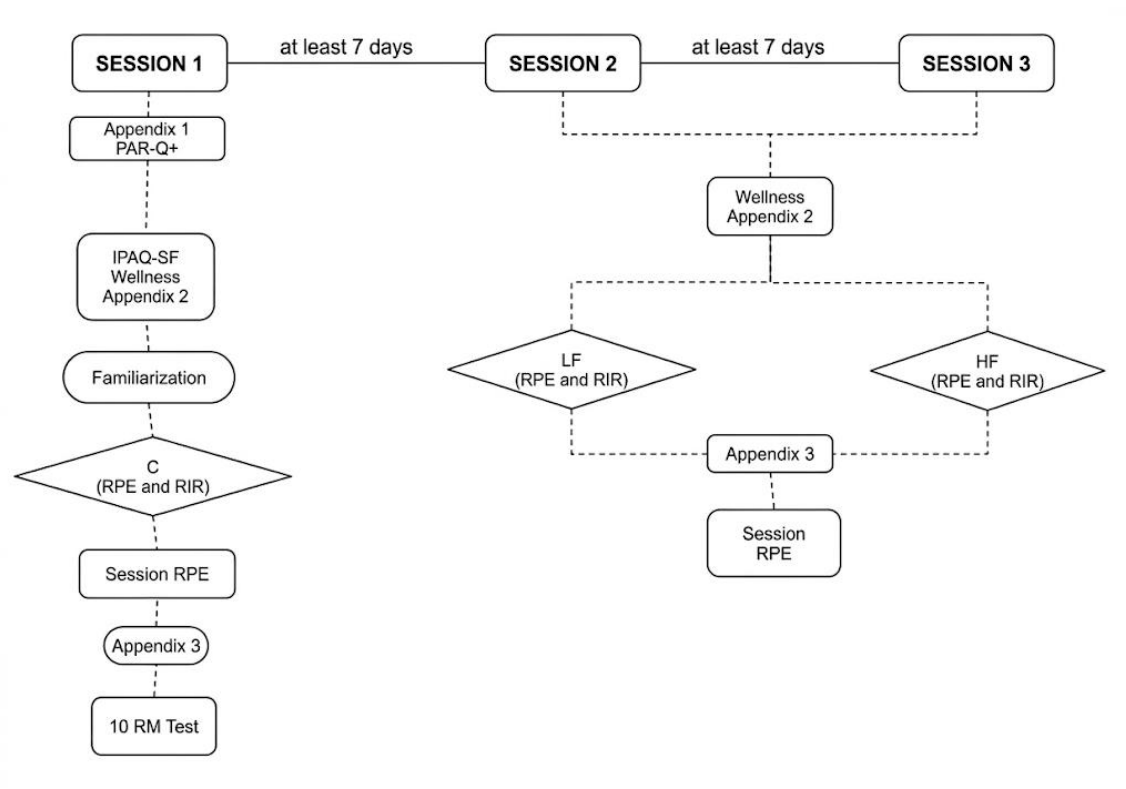


Figure 1: Development of the experimental protocol of the study sessions.

10RM: Maximum external load for 10 repetitions of the exercise, HF: High Fatigue exercise session, Annex 1: Information sheet and Informed Consent, Annex 2: Questionnaire on descriptive variables, Annex 3: Questionnaire to verify blinding, LF: Low Fatigue exercise session, C: Control exercise session, IPAQ-SF: short version of the International Physical Activity Questionnaire, PAR-Q+: Physical Activity Readiness Questionnaire, RIR: Repetitions in Reserve, RPE: Rate of Perceived Exertion.

- **Development of the intervention for session 1 (Session C):**

- Information about the study, informed consent, PAR-Q+ questionnaire, descriptive variables questionnaire, and IPAQ-SF questionnaire.
- Wellness Questionnaire.
- Anthropometric measurements.
- Familiarization with RPE and RIR measurements.
- Pre-intervention algometry.
- Pre-intervention isometric strength measurement.

- Warm-up (No external load): 2 sets x 15 repetitions with 120 seconds of rest after each set.
- LF protocol without load, with monitoring of RPE and RIR after each set.
- Immediate post-intervention algometry.
- Immediate post-intervention isometric strength measurement.
- Post-30min algometry.
- Post-30min isometric strength measurement.
- Perception of session fatigue: RPE of the complete session. 30 minutes after the intervention.
- 10-RM Test.
- **Development of the intervention for session 2 (Session HF):**
 - Wellness Questionnaire.
 - Pre-intervention algometry.
 - Pre-intervention isometric strength measurement.
 - Warm-up: 2 sets x 15 repetitions x 50% 10RM with 120 seconds of rest after each set.
 - HF Protocol: 3 sets x 10 repetitions x 100% 10RM with 180 seconds of rest between sets. With monitoring of RPE and RIR after each set.
 - Immediate post-intervention algometry.
 - Immediate post-intervention isometric strength measurement.
 - Post-30min algometry.
 - Post-30min isometric strength measurement.
 - Perception of session fatigue: RPE of the complete session. 30 minutes after the intervention.
- **Development of the intervention for session 3 (Session LF):**
 - Wellness Questionnaire.
 - Pre-intervention algometry.

- Pre-intervention isometric strength measurement.
- Warm-up: 2 sets x 15 repetitions x 50% 10RM with 120 seconds of rest after each set.
- LF Protocol: 6 sets x 5 repetitions x 100% 10RM with 180 seconds of rest between sets. With monitoring of RPE and RIR after each set.
- Immediate post-intervention algometry.
- Immediate post-intervention isometric strength measurement.
- Post-30min algometry.
- Post-30min isometric strength measurement.
- Perception of session fatigue: RPE of the complete session. 30 minutes after the intervention.

Regarding the evaluation of the outcome variables, we measure:

1. Pressure pain thresholds and pressure-evoked pain

Both variables will be evaluated using an analog manual algometer (Baseline Dolorimeter, MVS In Motion SL, Belgium). These measurements will be carried out at a local level (on one of the thighs, over the rectus femoris of the quadriceps, at the midpoint between the surface of the pelvis and the patella) and at a distance (on the neck over the belly of one of the upper trapezius muscles, at the midpoint between the surface of C7 and the acromion).

For the PPTs, incremental pressure will be exerted at a ratio of 1kg/s and the patient will be instructed to notify as quickly as possible upon "the minimum sensation of pain". The kilograms of pressure exerted to produce the minimum painful sensation will be the outcome variable. On the other hand, to evaluate the PEP, a fixed pressure of 6 kg will be applied and the intensity of the pain perceived by the subject will be collected according to the Numeric Rating Scale (NRS) verbally. The order of performance will always be the same, first PPT and then PEP. 3 repeated measurements will be carried out on 3 occasions throughout the session and we will take the average to reduce the variability of the measurement.

Since previous studies calculate EIH as the pre-post change in PPTs after the exercise intervention, in this project we evaluate the possible response in the same way (29,40).

2. Perceived exertion:

The monitoring of perceived exertion will be carried out with the OMNI-RES scales (OMNI-Resistance exercise Scale) as a measure of RPE for each set, and sRPE (Session rating of perceived exertion), as a measure of RPE for the complete session (41–43). The control of subjective effort will be carried out after each set and taking into account the OMNI-RES of each set (OMNI-RES1, OMNI-RES2...) and of the session, using the sRPE scale for this, as these are standardized instruments with greater reproducibility (43,44). As complementary data, the measurement of Repetitions in Reserve (RIR) will be used using the ERF scale (Estimated Repetitions to Failure scale) (45). For this, subjects will be asked how many more repetitions they believe could have been performed after each set. Although it has certain limitations because it is dependent on the subject's experience performing strength training (46), it can be a clinically interesting tool for internal load control. RPE (OMNI-RES) and RIR (ERF) for each set will be monitored immediately after each set, while sRPE will be monitored 30 minutes after the last set so that the effort of this last set does not interfere with the perception of effort of the global session.

3. Muscle fatigue (or Fatigability):

It will be monitored taking into account objective variables (not dependent on the patient's subjective report), through isometric knee extension action tests measured by dynamometry using a portable load cell (Model 620 Tedeo-Huntleigh, Vishay Precision Group Inc., Holon, Israel) and Chronojump software (Chronojump Bosco System, Barcelona, Spain) similar to the strategy used in other studies (47,48), as this has been shown to be a reliable measure. The variables we will use are the maximal voluntary isometric contraction (MVIC) measured in Newtons (N) and the Rate of Force Development (RFD) which calculates the slope of the force curve with respect to time ($\Delta\text{Force}/\Delta\text{time}$) expressed in ($\text{N}\cdot\text{s}^{-1}$) (47,48). These variables have proven to be sensitive to fatigue due to their evaluation of neuromuscular determinants (5,47). Furthermore, previous studies demonstrated reductions in the ability to generate force in the knee extensors upon the onset of fatigue, decreasing both peak force and the ability to produce force rapidly (5,49). Taking the pre-intervention MVIC and RFD values as a baseline, fatigability will be identified as a reduction in these post-intervention values.

Subjects will perform 2 valid repetitions (if there is not a difference of more than 20% between them), otherwise 3 repetitions will be performed, and the average of the 2 most similar repetitions will be used for analysis. The repetitions will consist of isometric contractions of 3-5 seconds under the premise “Contract as hard and fast as you can”.

Isometric strength (Muscle fatigue) and algometry (PPT and PEP) measurements will be carried out in the 3 experimental sessions in the same way. The body side on which each measurement will be performed will be determined randomly using proprietary software developed in R. 3 measurements will be performed in each session: pre, immediately post-intervention, and 30 minutes post-intervention.

On the other hand, intra-intervention perceived exertion measurements (RPE and RIR) will be carried out immediately after each set of exercise performed. The measurement of global perceived exertion for the session (sRPE scale) will be carried out 30 minutes post-intervention.

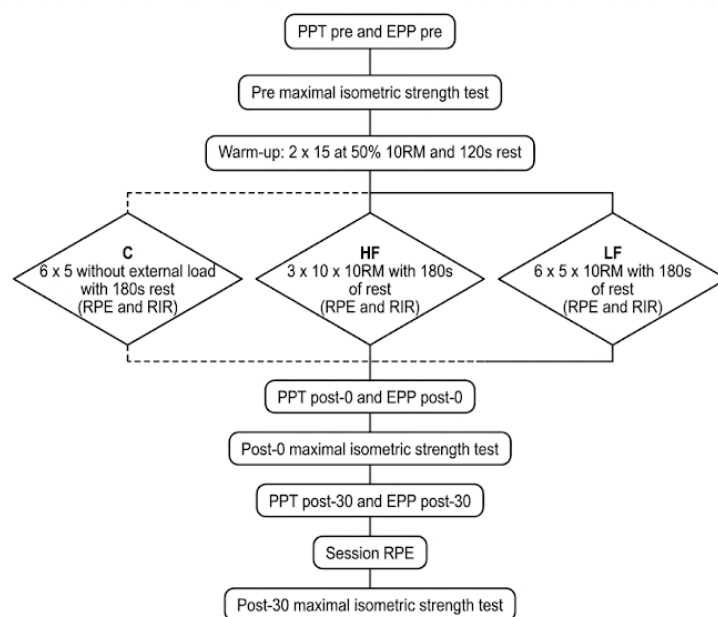


Figure 2: Development of outcome variable measurements

10RM: maximum load for 10 repetitions, HF: High Fatigue Exercise Session, LF: Low Fatigue Exercise Session, C: Control Exercise Session, RIR: Repetitions in Reserve, RPE: Rate of Perceived Exertion, PEP: pressure-evoked pain, post-0: immediately after the intervention, post-30: 30 minutes after the intervention, PPT: Pressure Pain Threshold, pre: immediately before the intervention.

EXPECTED ADVERSE EVENTS

Leg extension is a non-impact strength exercise performed with full stability due to the action of the guided movement path of the machine; therefore, it is highly unlikely that a moderate-to-severe adverse event will occur in healthy subjects.

One of the possible expected adverse events derived from performing this type of exercise is delayed onset muscle soreness, or DOMS. It manifests as muscular discomfort appearing between 24–48 hours after performing intense physical activity to which one is not conditioned. The appearance of this phenomenon does not pose a risk to the integrity of the participating subjects and does not require any type of therapeutic action, as it is a physiological mechanism related to exercise and recovery is spontaneous within 48 hours.

Other phenomena that may appear occasionally in certain subjects include sweating, tremors, dizziness, or fainting due to intense effort during physical exercise. Since participants are seated, there is no risk of falling, and they will be assisted with water and snack bars in case of vagal symptoms. Exceptionally, as a risk related to intense physical exercise, musculoskeletal injuries could occur; however, due to exercise control and programming, their appearance is highly unlikely. In the event of a minor musculoskeletal injury, current advice on its management will be provided, and conservative measures for inflammation—PEACE AND LOVE (50)—will be applied. In the event of a moderate musculoskeletal injury, the subject would be immediately transferred to the nearest medical center to receive appropriate healthcare.

Furthermore, all subjects will be provided with a telephone number and an email address where they can contact the project manager and principal investigator regarding any questions or setbacks.

PATIENT INFORMED CONSENT

Attached separately (see Annex 1).

PRACTICAL CONSIDERATIONS

Considering the total duration of the study, we establish a retention period for the study documentation of 3 years from its commencement. Accordingly, all collected data will be used solely for the purposes of this study, and information will be handled in accordance with current data protection regulations.

STATISTICAL ANALYSIS

The normality of the variables will be checked using the Shapiro-Wilk test and the graphical representation of the data in histograms and box plots. Regarding all analyses, parametric tests will be performed if the assumption of normality is met, and the corresponding non-parametric tests if it is not.

First, a descriptive analysis of the sample will be performed, attempting to summarize the data obtained, including absolute values (i.e., mean \pm standard deviation [SD]) and relative values (i.e., percentages, mean difference, etc.). Second, an inference analysis will be carried out. We will compare the different levels of fatigue (objective and subjective) obtained in the different sessions to confirm whether the protocols were effectively high and low fatigue. These comparisons will be made using a repeated measures ANOVA across the 3 sessions and specifically a paired t-test comparison between the high and low fatigue sessions to confirm if they are specifically different. To determine if the EIH phenomenon has indeed occurred, comparisons of the pre-post intrasession sensory thresholds will be made using paired t-tests.

To better establish the possible relationship between fatigue and EIH, we will perform a regression model to predict how much of the observed variability in the EIH effect is produced by fatigue (objective and subjective). Secondly, we will correlate fatigue measured objectively vs. subjectively using a Pearson correlation.

To determine if the level of prior physical activity, familiarity with training, and the general state in which patients arrive at the session (variables obtained through the Wellness questionnaire) are related to the magnitude of EIH and the onset of fatigue during exercise (specific objectives 1 and 2), they will be included in the regression model.

To determine if fatigability and perceived exertion are related to the descriptive variables of the sample (i.e., age, physical activity level, sleep-related variables, etc.) (specific objective 3), it will be explored using Pearson's correlation (or Spearman's, if necessary). We will secondarily explore whether the subjects' sex influences the onset or magnitude of EIH.

STARTING HYPOTHESIS

The starting hypothesis assumes that the fatigue achieved with resistance exercise has a directly proportional relationship with EIH. Therefore, it is believed that, although both exercise protocols may produce a state of EIH in the subjects, the HF exercise protocol, which should present a higher level of fatigue, will also induce greater EIH.

ENDPOINTS

The study endpoints are reflected below:

- Voluntary withdrawal of informed consent.
- Appearance of any pathology involving pain or medication.
- Any professional and/or personal setback that prevents attendance.
- Failure to comply with inclusion and/or exclusion criteria during participation.
- Appearance of pain during the sessions.

SAMPLE SIZE JUSTIFICATION

For the present sample size calculation, using the repeated measures ANOVA test as a reference: the variable used will be the pressure pain threshold measured with algometry, in which we assume an effect size of 0.11 (partial eta squared) derived from the most recent and similar work (26). Assuming a random error of 5% and a minimum statistical power of 80%, a sample size of 24 subjects has been calculated. Taking into account possible dropouts that may occur during the study (10%), this number has finally been increased to 27 subjects.

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ANNEX 1: PATIENT INFORMATION SHEET

STUDY TITLE: Hypoalgesic effects of strength exercise-induced fatigue in healthy people.

PRINCIPAL INVESTIGATOR: Miguel Delicado Miralles. Tel: 679788377 ; e-mail: mdelicado@umh.es.

CENTER: Miguel Hernández University

INTRODUCTION

We are contacting you to inform you about a research study in which you are invited to participate. The study has been approved by a Drug Research Ethics Committee, in accordance with current legislation. Our intention is only for you to receive correct and sufficient information so that you can evaluate and judge whether or not you want to participate in this study. To do this, read this information sheet carefully and we will clarify any doubts that may arise after the explanation. Additionally, you can consult with the people you deem appropriate.

VOLUNTARY PARTICIPATION

You should know that your participation in this study is voluntary and that you can decide not to participate or change your decision and withdraw consent at any time, without any prejudice to you.

GENERAL DESCRIPTION OF THE STUDY

Strength exercise is an active and dynamic exercise that consists of applying force against a resistance. This produces fatigue, which is observed as a reduction in the ability to apply force during and after an exercise, and perception of effort, which is an individual subjective assessment. Strength exercise can also be used for therapeutic purposes, as it presents a great diversity of beneficial effects on different body systems and apparatuses. One of them is the modulation of pain perception; it has already been seen that performing strength exercises can reduce

the perception of pain, a phenomenon known as exercise-induced hypoalgesia (EIH), although the exact physiological mechanisms involved or the dose necessary to produce this effect are not known.

One of the exercises on which there are most studies in this line is the leg extension exercise in a machine (known as *leg extension*), which is an analytical exercise, in an open kinetic chain, performed guided by the action of the machine itself, which allows for greater stability and minimizes the impact of other variables during exercise. This is generally used to strengthen and hypertrophy the anterior musculature of the thigh, especially the quadriceps femoris, but it can also be used to evaluate the effects on EIH.

- **Main Objective:** To determine if fatigue and perceived effort in strength exercise are a relevant factor for the appearance of EIH in healthy people.
- **Procedure:** Participants will perform 3 sessions separated by at least 7 days, involving a control protocol and 2 protocols intended to differentiate different levels of fatigue.
- **Measurements:** Before, during, and after the protocols, measures of algometry, isometric strength, and perceived effort will be taken.
- **Additional Tests:** A maximum weight test for 10 repetitions and various questionnaires will be conducted to classify participants by descriptive variables (sex, age, height...) and evaluate weekly physical activity, familiarity with strength exercise, prior fatigue, sleep quality, general muscle state, pain, stress levels, and mood.
- **Anonymity:** All data will be completely anonymous when publishing the results of this study.

BENEFITS AND RISKS DERIVED FROM YOUR PARTICIPATION IN THE STUDY

You will not receive any financial compensation. You could obtain some benefit associated with the benefits of strength exercise. However, it is possible that you will

not obtain any health benefit from participating in this study. This study aims to improve knowledge about exercise-induced hypoalgesia and its relationship with fatigue to improve physical exercise recommendations.

Inconveniences and risks:

- You would have to come a total of 3 times for measurements and interventions. A total of three sessions will be performed.
- Secondary adverse risks, with a maximum duration of around 48-72 hours, are as follows:
 - o Delayed onset muscle soreness (DOMS): due to physical exercise.
 - o These events, which do not require medical or pharmacological intervention, resolve spontaneously and do not cause disability beyond mild discomfort.
 - o If effects last more than 72 hours or are more intense than expected, they will be treated with conservative physical therapy or referred to a specialist.
- Very unlikely side effects include:
 - o Sweating, tremors, dizziness, or fainting: as a vasovagal response to intense effort during exercise.
 - o Musculoskeletal injuries as a risk related to intense physical exercise.

In these cases, the subject would be moved to the nearest medical center immediately.

A telephone number and email will be provided to contact the project manager and principal investigator regarding any doubts or setbacks.

CONFIDENTIALITY

The treatment and communication of personal data will comply with Organic Law 3/2018 and EU Regulation 2016/679. Data will be collected in a research file exclusively for this study. You may exercise rights of access, rectification, opposition, and cancellation by contacting the study manager. Data will be identified by a code; only the investigator/collaborators can relate this data to you. Your identity will not be revealed except in medical emergencies or legal requirements. Access is restricted to authorized personnel (investigators, health authorities, Ethics Committee) to check data and procedures. Data transmitted to third parties or other countries will never contain directly identifying information (name, address, etc.).

FINANCIAL COMPENSATION

This study does not contemplate financial remuneration.

OTHER RELEVANT INFORMATION

Any new information regarding EIH that may affect your willingness to participate will be communicated as soon as possible. If you withdraw consent, no new data will be added, and you can demand the destruction of identifiable samples. You may be excluded from the study by the promoter/investigators for safety reasons or non-compliance. In either case, you will receive an explanation for your withdrawal. By signing, you agree to comply with study procedures. You will receive the best available treatment during and after the study.

PARTICIPANT CONSENT FORM

Study Title: Hypoalgesic effects of strength exercise-induced fatigue in healthy people.

I, (name and surname of the participant)
.....

- I have read and understood the information sheet provided to me about the study.
- I have been able to ask questions about the study.
- I have received sufficient information about the study.
- I have spoken with:
- I know and assume the risks and/or consequences that could derive from my participation.
- I understand that I can withdraw from the study whenever I want without having to give explanations.
- In accordance with Organic Law 3/2018 and EU Regulation 2016/679, I declare that I have been informed of the existence of a personal data file, its purpose, and the recipients of the information.
- I freely provide my agreement to participate in the study.

Participant's Signature

|

Investigator's Signature

Date: //__

|

Date: //__

REVOCATION OF CONSENT

I, Mr./Ms., with ID:.....

REVOKE the consent previously given for the performance of this procedure of my own free will, and I assume the consequences derived therefrom.

Patient's Signature

Date //__

ANNEX 2: QUESTIONNAIRE ON DESCRIPTIVE VARIABLES

SESSION 1

Participant Code: _____

To be filled out by the participant:

- Sex: _____
- Age: _____
- Height (in cm): _____
- Weight (in kg): _____
- Are you currently in pain? _____
- Have you had episodes of chronic pain (lasting more than 3 months) in the last 6 months? _____
- Do you currently have any pathology? If yes, please specify: _____
- Do you have symptoms of any kind associated with any pathology? If yes, please specify: _____

- Have you consumed analgesic, antipyretic, non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, opioids, or similar in the last 24 hours?

- If yes, please specify: _____
- Have you consumed drugs of any nature or food supplements in the last 24 hours? If yes, please specify: _____
- Have you consumed alcohol or other psychoactive substances in the last 24 hours? If yes, please specify: _____
- Have you consumed coffee or other stimulants in the last 24 hours? If yes, please specify: _____
- Do you consider yourself a physically active person? _____
- Do you perform strength exercise sessions? _____
- How many times per week do you perform strength exercise sessions?

- How would you describe the intensity of your strength exercises on a scale of 0 to 10? (0 being an extremely easy workout and 10 being an extremely hard workout) _____
- How many days per week do you perform strength exercises?

- How long do your strength exercises usually last? (in minutes)

Mark the most accurate response with an X

1. Regarding strength exercise performed with a high perception of subjective effort and which produces high fatigability...

- ☐ It is very dangerous and potentially painful for me.
- ☐ It is not dangerous at all, nor can it cause me pain.
- ☐ It can become dangerous and cause me pain.
- ☐ I am not sure if it is dangerous and can cause me pain.
- ☐ It is not dangerous, nor do I think it can cause me pain in most cases.

2. Regarding the level of concern or fear produced by performing strength exercise with a high perception of subjective effort or high fatigability...

- ☐ I am very worried about hurting myself and feeling pain while performing these types of exercises; I always try to avoid them.
- ☐ I am worried about hurting myself and feeling pain while performing these types of exercises; I prefer to avoid them, but I can try to do them.
- ☐ I might be a little worried about hurting myself and feeling pain while performing these types of exercises, but I perform them.
- ☐ I am not too worried about hurting myself and feeling pain while performing these types of exercises; I perform them regularly, although they are not my favorite.
- ☐ I am not worried at all about hurting myself and feeling pain while performing these types of exercises; I perform them regularly.

To be filled out by the examiner:

| |
|---------------------------|
| Lower limb length (in cm) |
| |

SESSION 2

Participant Code: _____

- Are you currently in pain? _____
- Have you had episodes of chronic pain (lasting more than 3 months) in the last 6 months? _____
- Do you currently have any pathology? If yes, please specify: _____
- Do you have symptoms of any kind associated with any pathology? If yes, please specify: _____
- Have you consumed analgesic, antipyretic, non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, opioids, or similar in the last 24 hours? _____
- If yes, please specify: _____
- Have you consumed drugs of any nature or food supplements in the last 24 hours? If yes, please specify: _____
- Have you consumed alcohol or other psychoactive substances in the last 24 hours? If yes, please specify: _____
- Have you consumed coffee or other stimulants in the last 24 hours? If yes, please specify: _____

SESSION 3

Participant Code: _____

- Are you currently in pain? _____
- Have you had episodes of chronic pain (lasting more than 3 months) in the last 6 months? _____

- Do you currently have any pathology? If yes, please specify:

- Do you have symptoms of any kind associated with any pathology? If yes, please specify: _____
- Have you consumed analgesic, antipyretic, non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, opioids, or similar in the last 24 hours?

- If yes, please specify: _____
- Have you consumed drugs of any nature or food supplements in the last 24 hours? If yes, please specify: _____
- Have you consumed alcohol or other psychoactive substances in the last 24 hours? If yes, please specify: _____
- Have you consumed coffee or other stimulants in the last 24 hours? If yes, please specify: _____

ANNEX 3: QUESTIONNAIRE ON BLINDING SUCCESS

SESSION 1

Identification Code: _____

Given that this study includes a control session and 2 different interventions in which high and low fatigue exercise protocols will be performed, please mark the option you believe to be most correct with an X:

In session 1...

- ☐ I am sure that I performed the control protocol.

- ☐ I think I performed the control protocol.
- ☐ I am sure that I performed the high fatigue protocol.
- ☐ I think I performed the high fatigue protocol.
- ☐ I am sure that I performed the low fatigue protocol.
- ☐ I think I performed the low fatigue protocol.
- ☐ I am not sure which protocol I performed.

To be filled out by the examiner: Session 1 corresponded to the protocol of:

SESSION 2

Identification Code: _____

Given that this study includes a control session and 2 different interventions in which high and low fatigue exercise protocols will be performed, please mark the option you believe to be most correct with an X:

In session 2...

- ☐ I am sure that I performed the control protocol.
- ☐ I think I performed the control protocol.
- ☐ I am sure that I performed the high fatigue protocol.
- ☐ I think I performed the high fatigue protocol.
- ☐ I am sure that I performed the low fatigue protocol.
- ☐ I think I performed the low fatigue protocol.

- ☐ I am not sure which protocol I performed.

To be filled out by the examiner: Session 2 corresponded to the protocol of:

SESSION 3

Identification Code: _____

Given that this study includes a control session and 2 different interventions in which high and low fatigue exercise protocols will be performed, please mark the option you believe to be most correct with an X:

In session 3...

- ☐ I am sure that I performed the control protocol.
- ☐ I think I performed the control protocol.
- ☐ I am sure that I performed the high fatigue protocol.
- ☐ I think I performed the high fatigue protocol.
- ☐ I am sure that I performed the low fatigue protocol.
- ☐ I think I performed the low fatigue protocol.
- ☐ I am not sure which protocol I performed.

To be filled out by the examiner: Session 3 corresponded to the protocol of:
